

U.S. DEPARTMENT OF COMMERCE
National Technical Information Service
PB-299 299

Effects of Environmental Factors on Radiocadmium Uptake by Four Species of Marine Bivalves

(U.S.) Environmental Research Lab, Narragansett, RI

5 Nov 76

TECHNICAL REPORT DATA (Please read instructions on the reverse before completing)		
1. REPORT NO. EPA-600/J-77-158	2. JOURNAL ARTICLE	3. PB 299299
4. TITLE AND SUBTITLE Effects of Environmental Factors on Radiocadmium Uptake by Four Species of Marine Bivalves		5. REPORT DATE 1977 (Issue Date)
		6. PERFORMING ORGANIZATION CODE
7. AUTHOR(S) E. Jackim, G. Morrison, and R. Steele		8. PERFORMING ORGANIZATION REPORT NO.
9. PERFORMING ORGANIZATION NAME AND ADDRESS U.S. Environmental Protection Agency Environmental Research Laboratory Narragansett, RI 02882		10. PROGRAM ELEMENT NO.
		11. CONTRACT/GRANT NO.
12. SPONSORING AGENCY NAME AND ADDRESS U.S. Environmental Protection Agency Environmental Research Laboratory Narragansett, RI 02882		13. TYPE OF REPORT AND PERIOD COVERED journal reprint
		14. SPONSORING AGENCY CODE
15. SUPPLEMENTARY NOTES REFERENCE: MARINE BIOLOGY, 40:303-308, 1977		
16. ABSTRACT Temperature, salinity, bottom-sediment type, and zinc concentration all influenced Cd uptake by 4 marine bivalves (<i>Mya arenaria</i> , <i>Mytilus edulis</i> , <i>Mulinia lateralis</i> and <i>Nucula proxima</i>) in short-term static assay systems using ¹⁰⁹ Cd as a tracer. The experimental system consisted of aquaria containing 20 l of seawater maintained under controlled light and temperature conditions. The water contained either 5 or 20 µg/l Cd and tracer. Distribution and kinetics of the metal were monitored in the water column and organisms. The results demonstrate that Cd uptake rates differed widely among the organisms tested. An increase in temperature increased Cd uptake rate by all test organisms. A decrease in salinity increased Cd uptake by all organisms tested. The presence of bottom sediment depresses Cd accumulation in some benthic animals. Zinc in concentrations of 0.5 mg/l substantially decreased Cd uptake by <i>Mytilus edulis</i> and <i>Mulinia lateralis</i> . It is suggested that all important species and environmental variables be considered when studying heavy-metal uptake by marine organisms or when establishing water-quality criteria.		
17. KEY WORDS AND DOCUMENT ANALYSIS		
a. DESCRIPTORS	b. IDENTIFIERS/OPEN ENDED TERMS	c. COSATI Field/Group
Biological oceanography, Water Pollution and Control, Environment, Environmental health and safety		
18. DISTRIBUTION STATEMENT Release Unlimited	19. SECURITY CLASS (This Report) 20. SECURITY CLASS (This page)	21. NO. OF PAGES 8 Pages 22. PRICE A02-A01

Effects of Environmental Factors on Radiocadmium Uptake by Four Species of Marine Bivalves

E. Jackim, G. Morrison and R. Steele

Environmental Protection Agency, Environmental Research Laboratory; Narragansett, Rhode Island, USA

Abstract

Temperature, salinity, bottom-sediment type, and zinc concentration all influenced Cd uptake by 4 marine bivalves (*Mya arenaria*, *Mytilus edulis*, *Mulinia lateralis* and *Nucula proxima*) in short-term static assay systems using ^{109}Cd as a tracer. The experimental system consisted of aquaria containing 20 l of seawater maintained under controlled light and temperature conditions. The water contained either 5 or 20 $\mu\text{g/l}$ Cd and tracer. Distribution and kinetics of the metal were monitored in the water column and organisms. The results demonstrate that Cd uptake rates differed widely among the organisms tested. An increase in temperature increased Cd uptake rate by all test organisms. A decrease in salinity increased Cd uptake by all organisms tested. The presence of bottom sediment depresses Cd accumulation in some benthic animals. Zinc in concentrations of 0.5 mg/l substantially decreased Cd uptake by *Mytilus edulis* and *Mulinia lateralis*. It is suggested that all important species and environmental variables be considered when studying heavy-metal uptake by marine organisms or when establishing water-quality criteria.

Introduction

Many studies have been concluded concerning the fate and distribution of metals in marine ecosystems. Recently, Fowler and Benayoun (1974) explored Cd flux using radiocadmium in mussels and shrimp. The object of our work is to develop methods which can easily and quickly answer questions concerning metal uptake by organisms as affected by various environmental factors. This study explores Cd flux in several marine bivalves under the influence of selected environmental variables. These variables are: temperature, salinity, and sediment, as well as the influence of Zn.

Materials and Methods

Our test system was designed with an emphasis on simplicity. The test tanks were 40 l glass (60 x 30 x 30 cm) aquaria containing 20 l filtered seawater of desired salinity (Fig. 1). Seawater from Narragansett Bay was continuously filtered through calcium carbonate biological filters and aerated. Experiments exceeding 2 weeks duration necessitated

biweekly water changes to insure good water quality. Measurements of nitrate and phosphate were performed to determine the efficiency of the filtration system and frequency of water replacement. Continuous mixing was accomplished with a magnetic stirring bar. Pyrex dishes with or without sediment contained the test organisms. The tanks were placed in temperature and light-controlled environmental chambers. Non-radioactive cadmium chloride with $^{109}\text{CdCl}_2$ as a radioactive tracer was added to each of the experimental tanks. The specific activity of Cd in the tanks ranged between 18,600 to 24,000 counts per minute (cpm) per μg Cd or 0.016 to 0.02 $\mu\text{Ci}/\mu\text{g}$ Cd, depending upon the particular study. The final activity was approximately 0.1 $\mu\text{Ci/l}$ and a Cd concentration of 5 or 20 $\mu\text{g/l}$, depending on the experiment. Cadmium concentrations, measured as radioactivity, were held constant throughout each experiment by the addition of CdCl_2 of the same specific activity.

Animals used were the filter-feeding bivalves *Mya arenaria*, *Mytilus edulis*, *Mulinia lateralis*, and the deposit-feeding bivalve *Nucula proxima*. *Mya arenaria* and *Mytilus edulis*

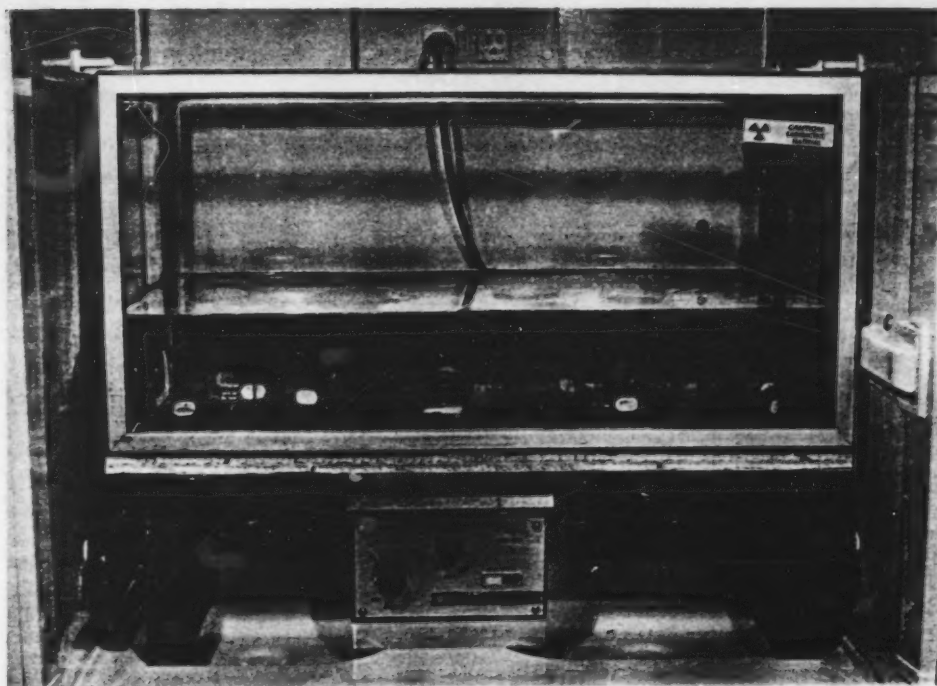


Fig. 1. One of 4 experimental aquarium tanks in which experimental bivalves were placed. Biological filters and aeration are not shown

Table 1. Cd uptake by marine bivalves. Mean Cd uptake ($\mu\text{g Cd/g}$ dry body-tissue) of 10 specimens of each species held at 10° and 20°C , 30‰ S, no sediment, and exposed to 5 and $20\ \mu\text{g/l}$ Cd for 14 days. Values between 10° and 20°C are significantly different between each species except *Mytilus edulis*. SD: Standard deviation; nd: no data

Species	5 $\mu\text{g Cd/l}$				20 $\mu\text{g Cd/l}$			
	10°C		20°C		10°C		20°C	
	$\bar{X}$	SD	$\bar{X}$	SD	$\bar{X}$	SD	$\bar{X}$	SD
<i>Mya arenaria</i>	2.2 $\pm$ 0.31		4.2 $\pm$ 0.25		16.76 $\pm$ 1.23		29.04 $\pm$ 0.94	
<i>Mytilus edulis</i>	9.3 $\pm$ 2.48		9.4 $\pm$ 0.91		50.55 $\pm$ 8.57		60.35 $\pm$ 11.8	
<i>Mulinia lateralis</i>	3.6 $\pm$ 0.21		8.9 $\pm$ 1.03		10.33 $\pm$ 0.27		20.46 $\pm$ -	
<i>Nucula proxima</i>	nd		nd		2.0 $\pm$ 1.13		4.56 $\pm$ 1.19	

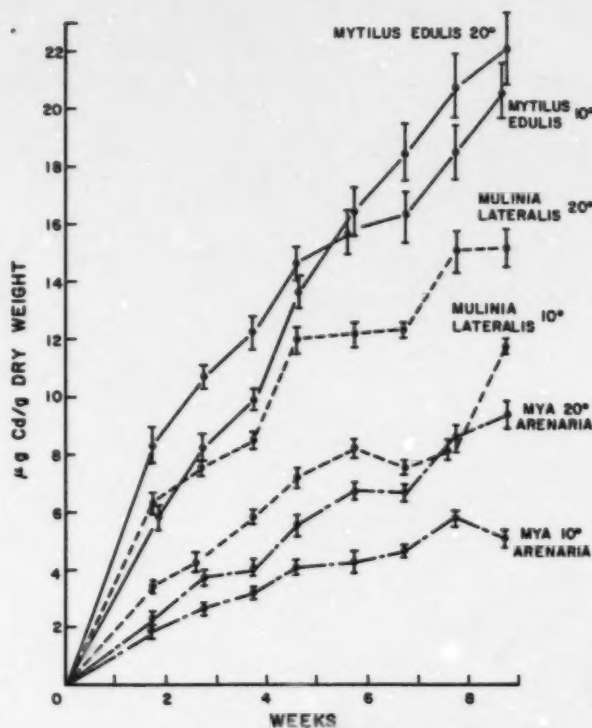


Fig. 2. Uptake of Cd by 3 bivalve species. Each point represents mean of 10 specimens. Cd concentration was maintained at 5 µg/l at 28‰ S. Bivalves were fed weekly and water was changed at 2-week intervals. No sediment was present. Bars represent standard errors

were juveniles with shell lengths ranging from 12 to 23 mm for both species. *Mulinia lateralis* shell length ranged from 8 to 14 mm, *Nucula proxima* averaged 3 mm in shell length. All organisms of a given species were collected at specific locations in local Rhode Island waters. They were held in the test aquaria under controlled conditions of temperature, salinity, light, and sediment for a 7 to 14-day acclimation period, specified for each experiment. All the organisms in a given experimental treatment were held by species in open 80 x 100 mm Pyrex dishes within an aquarium. The dishes contained sediment collected from Narragansett Bay for those experiments dealing with the effects of sediment type and presence on Cd uptake. The aquaria contained at least 10 to 15 bivalves of each species. Two types of sediment were used in these experiments.

¹ Mention of specific products herein does not constitute endorsement by the U.S. Environmental Protection Agency.

The sediment type referred to as mud was collected from Narragansett Bay and contained approximately 1.5% organic material. The sand substrate was collected from a beach and contained only 0.2% organic matter.

The effects of zinc on cadmium uptake were tested by adding specified amounts of unlabeled ZnCl₂ to the test tanks containing 5 µg/l labeled CdCl₂.

At weekly intervals, individual specimens were removed from the tanks and washed twice with filtered seawater. They were placed in individual plastic counting tubes and counted for a minimum of 2 min in a Packard Liquid Scintillation Counter¹ adapted for nondestructive measuring of gamma activity (Jackim, 1975). Counting efficiency was 53%. Shielding of radioactivity by the shell of the bivalves was insignificant. All specimens were returned to the aquaria immediately after counting. After the final sampling period, all test organisms were sacrificed, freeze-dried and weighed.

The results, except for saline and sediment studies, are reported as µg Cd accumulation per gram dry weight. Saline and sediment studies are reported as cpm per gram dry weight. Experimental results are reported as dry weight of soft tissue unless stated otherwise. Reported differences in results are significant at the 95% confidence interval level as examined with Duncan's Multiple Range Test (Steele and Torrie, 1960).

Results

Variable Cadmium Concentration and Temperature

The magnitude and rank-order of Cd uptake by the test organisms after 14 days exposure in seawater at 30‰ and 10‰ and 20°C is shown in Table 1. A marked species difference occurred, *Mytilus edulis* having an order of magnitude greater accumulation than *Nucula proxima*. The filter-feeders, as a group, accumulate significantly greater amounts of Cd than the deposit-feeder *N. proxima*.

The results of a 9-week exposure of the 3 filter feeding species to Cd at 10°C and 20°C indicate a relationship between uptake rates and body residue. The initial rate of Cd uptake decreases with increasing body burden (Fig. 2).

The effect of two temperature regimes (10°C and 20°C) on accumulation of Cd is shown in Table 1. It is apparent that some species exhibit a much greater temperature response than others. *Mytilus edulis*, for example, showed no signifi-

Table 2. Cd uptake by 3 marine bivalves at 10°C and 20°C with and without temperature acclimation. Each group of 10 specimens was acclimated for 2 weeks to specified temperature, then placed in test water containing 5 µg/l Cd for 6 days

Acclimation temperature (°C)	Test temperature (°C)	µg Cd/g (dry weight) upon 5 days Cd exposure					
		<i>Mya arenaria</i>		<i>Mytilus edulis</i>		<i>Mulinia lateralis</i>	
		$\bar{X}$	SD	$\bar{X}$	SD	$\bar{X}$	SD
20	20	12.97 ± 1.94		20.56 ± 1.03		5.87	ps
10	20	9.86 ± 0.29		18.09 ± 1.40		5.04	ps
20	10	7.67 ± 0.34		16.38 ± 2.42*		3.86 ± 0.81	
10	10	6.71 ± 0.67		21.56 ± 2.94*		2.79	ps

ps: Pooled samples.

*Differences statistically significant; P = 0.05.

Table 3. *Mya arenaria* and *Mulinia lateralis*. Radiocadmium accumulation (cpm) in bivalves exposed to 5 µg/l Cd for 7 days in dishes containing no sediment, sand, and bottom sediment at 10°C and 28‰ S

Conditions	<i>Mya arenaria</i>		<i>Mulinia lateralis</i>	
	$\bar{X}$	SD	$\bar{X}$	SD
No sediment	6111 ± 985		2294 ± 743	
Sand	4730 ± 1057		2265 ± 793	
Mud	2881 ± 515		1496 ± 732	
F test	32.24, all treatments significantly different		2.99, no significant difference between treatments	

Table 4. Influence of salinity on Cd uptake (µg Cd/g dry tissue) in soft tissue of 3 marine bivalves at 10°C and 20°C. Bivalves were exposed to 20 µg/l Cd for 21 days. Results are mean values of pooled sample of at least 10 specimens

Species	10°C		20°C	
	20‰ S	30‰ S	20‰ S	30‰ S
<i>Mytilus edulis</i>	83.16	32.08	108.12	86.62
<i>Mulinia lateralis</i>	52.27	24.35	36.91	8.78
<i>Nucula proxima</i>	2.08	0.61	5.35	2.61

cant difference in Cd uptake between 10°C and 20°C at 5 µg and 20 µg Cd/l, respectively. *Mulinia lateralis* at 20°C (20 µg Cd/l) displayed a 90% increase over *M. lateralis* held at 10°C. Temperature-acclimation studies indicated that acclimation within 10°C to 20°C had little, if any, influence on Cd uptake by *Mya arenaria* and *Mulinia lateralis* (Table 2). *Mytilus edulis* initially exhibited a greater accumulation rate in 10°C-acclimated specimens tested at 10°C than those acclimated at 20°C and tested at 10°C. Accumulation was comparable in *Mya arenaria* and *Mulinia lateralis* acclimated at 20°C for 2 weeks

and tested at 10°C to those acclimated and tested at 10°C. Likewise, bivalves acclimated for 2 weeks at 10°C and transferred to 20°C exhibited the same uptake as those acclimated and tested at 20°C (Table 2).

Sediment Type

Sediment types were found to influence the rate of Cd accumulation by *Mya arenaria*. This organism took up the greatest amount when held without a substrate and progressively less when held in sand and mud sediments (Table 3). Although the same trend was evident with *Mulinia lateralis*, the difference was not statistically significant.

Presence of Zinc

The addition of 500 and 1000 µg/l Zn to seawater containing 5 µg/l Cd decreased Cd uptake by *Mytilus edulis* and *Mulinia lateralis* with respect to controls (Fig. 3).

Salinity

A salinity decrease from 30 to 20‰ produced a 24 to 400% increase in Cd uptake at 10°C and 20°C in all bivalves tested (Table 4).

Discussion

Previous studies have shown that Cd uptake rates by various marine organisms are correlated with Cd concentration in the water (Hiyama and Shimizu, 1964; O'Hara, 1973; Eisler, 1974 and Fowler and Benayoun, 1974) and the type of organism (vertebrate, invertebrate, algal) examined (Eisler et al., 1972). Species within a given taxonomic group but with different environmental habitats exhibit widely varying Cd accumulation rates. For example, *Mytilus edulis* accumulates Cd nearly four times faster than *Mya arenaria*. Feeding type within a group also appears to play a significant role, with the infaunal filter-feeder *Mulinia lateralis* accumulating about 5 times more Cd than the deposit-feeder *Nucula proxima* (Table 1).

The evidence presented in this study suggests that early uptake rates might be indicative of subsequently acquired body burdens after long-term exposure (Fig. 2). The best test of this hypothesis would be a field study or a long-term flow-through laboratory study using

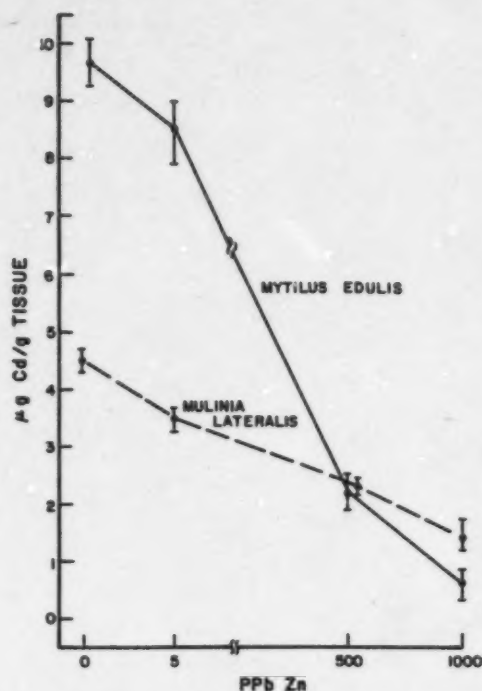


Fig. 3. Effect of Zn on Cd uptake by 2 bivalve species. Organisms exposed to 5 µg/l Cd with added increments of Zn at 10°C and 28‰ S for 7 days. Each point is mean value for 10 specimens. Bars represent standard errors

several species having different habitats (epifaunal, infaunal) and feeding types.

Generally it is assumed that metal uptake increases as temperature increases within a normal metabolic range, but the present work (Table 1) and that of Fowler and Benayoun (1974) indicate that this hypothesis is not necessarily correct. Neither study demonstrated any significant influence of temperature on Cd uptake by two species of *Mytilus* during winter months, although our summer studies (Table 4) did show a temperature response by *Mytilus edulis*. Experiments in which winter organisms were acclimated to 10°C and transferred to 20°C (and vice versa) showed Cd uptake values comparable to those acclimated and held at each test temperature. It appears that prior thermal history has little influence on metal accumulation. Nevertheless, temperature remains a variable to be considered when considering the impact of Cd pollution on a marine ecosystem.

Parizek (1957) suggested a physiological competition between Zn and Cd in mice. Eisler and Gardner (1973), using a

rather high concentration of both Zn (36 mg/l) and Cd (10 mg/l), found that the two metals had an additive effect with regard to toxicity in *Fundulus heteroclitus*, but that Cd decreased Zn uptake in that study. The present study showed that 5 µg/l Zn tended to decrease Cd uptake in *Mytilus edulis* and *Mulinia lateralis* although this trend was not statistically significant. At 500 µg/l Zn, the decrease was highly significant. Studies by Fowler and Benayoun (1974) showed a trend toward decreased Cd uptake at 100 µg/l Zn by *Mytilus galloprovincialis*, but this was not statistically significant. Taken together, this evidence indicates that Zn does inhibit Cd uptake in some species but not in others. This competition is probably not very significant from the ecological viewpoint because of the high levels of Zn required to reduce Cd uptake in seawater. Nevertheless, the physiological mechanism of metal interaction remains of interest.

Our finding that sediments decrease Cd uptake in various benthic organisms is important to consider in future laboratory studies. Preliminary experiments indicate that a Cd concentration gradient is formed at the sediment-water interface in our test systems, and that bivalves tend to filter slower when in sediment. The relative importance of these two factors in depressing Cd uptake in natural environments, however, is unknown.

The evidence indicates that a lower salinity favors greater Cd accumulation, thus salinity is an important environmental variable which should be considered when establishing water-quality criteria both from the viewpoint of tissue concentration and toxicity.

This study indicates that several environmental factors (e.g. temperature, salinity, composition of bottom sediment, and the presence of another metal, Zn) influence Cd uptake rates. The observed differences in Cd uptake are of sufficient magnitude to be of some ecological significance and should be considered when establishing water-quality criteria. Field studies and long-term laboratory flow-through studies are needed to assess the significance of these findings in the marine environment.

Acknowledgements. Thanks are due to Mr. F. Osterman and Ms. K. Bonatti for technical assistance in this study.

Literature Cited

- Eisler, R.: Radiocadmium exchange with seawater by *Fundulus heteroclitus* (L.). Fish Biol. 6, 601-612 (1974)

- and G.R. Gardner: Acute toxicity to an estuarine teleost of mixtures of cadmium, copper and zinc salts. *J. Fish Biol.* 5, 131-142 (1973)
- , G.E. Zarogian and R.J. Hennekey: Cadmium uptake by marine organisms. *J. Fish. Res. Bd Can.* 29, 1367-1369 (1972)
- Fowler, S.W. and G. Benayoun: Experimental studies on cadmium flux through marine biota. In: Comparative studies of food and environmental contamination, pp 159-178. Vienna: International Atomic Energy Agency 1974
- Hiyama, Y. and M. Shimizu: On the concentration factors of radioactive Cs, Sr, Cd, Zn and Ce in marine organisms. *Rec. oceanogr. Wks Japan* 7, 43-77 (1964)
- Jackim, E.: A simple method for providing liquid scintillation counters with external fluor, well counting capabilities. *Analyt. Biochem.* 63, 630-633 (1975)
- O'Hara, J.: The influence of temperature and salinity on the toxicity of cadmium to the fiddler crab, *Uca pugilator*. *Fish. Bull. U.S.* 71, 149-153 (1973)
- Parizek, J.: The destructive effect of cadmium ion on testicular tissue and its prevention by zinc. *J. Endocr.* 15, 56-63 (1957)
- Steele, R.G.D. and J.H. Torrie: Principles and procedures of statistics, 481 pp. New York: McGraw-Hill, Inc. 1960
- Dr. Eugene H. Jackim
Environmental Research
Laboratory, EPA
S. Ferry Rd.
Narragansett, Rhode Island
02882
USA

Date of final manuscript acceptance: November 5, 1976. Communicated by M.R. Tripp, Newark